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☐ 1: Clin Ther 1999 Oct;21(10):1653-63

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FULL-TEXT ARTICLE

## Comparison of rofecoxib and celecoxib, two cyclooxygenase-2 inhibitors, in postoperative dental pain: a randomized, placebo- and active-comparator-controlled clinical trial.

**Malmstrom K, Daniels S, Kotey P, Seidenberg BC, Desjardins PJ.**

Pulmonary-Immunology Department, Merck Research Laboratories, Rahway, New Jersey, USA.

Pain is a common complaint, often occurring in conjunction with inflammation. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used analgesic agents in ambulatory patients. In recent studies, the cyclooxygenase-2 (COX-2) inhibitor rofecoxib demonstrated analgesic effects similar to those of NSAIDs in the treatment of acute pain and primary dysmenorrhea. The present randomized, single-dose, double-blind, double-dummy, placebo- and active-comparator-controlled, parallel-group study was undertaken to compare the analgesic efficacy of the COX-2 inhibitors rofecoxib 50 mg and celecoxib 200 mg with that of ibuprofen 400 mg and placebo in patients with postoperative dental pain. Two hundred and seventy-two patients experiencing pain after the removal of  $\geq 2$  third molars were randomized according to pain severity (moderate vs severe) to receive a single dose of placebo ( $n = 45$ ), rofecoxib 50 mg ( $n = 90$ ), celecoxib 200 mg ( $n = 91$ ), or ibuprofen 400 mg ( $n = 46$ ). Using a patient diary, patients recorded pain intensity, pain relief, and global evaluations throughout the 24-hour period after dosing. The overall analgesic effect, onset of action, peak effect, and duration of effect were evaluated, with the primary end point being total pain relief over 8 hours (TOPAR8). The safety profile was assessed on the basis of physical findings, laboratory results, and spontaneous reports of adverse experiences. The results showed that compared with celecoxib, rofecoxib had superior analgesic effects on all measures of analgesic efficacy, including overall analgesic effect (TOPAR8, 18.3 vs. 12.5;  $P < 0.001$ ), time to onset of effect (30 vs. 60 minutes;  $P = 0.003$ ), peak pain relief (score, 2.8 vs 2.3;  $P < 0.05$ ), and duration of effect ( $> 24$  vs. 5.1 hours;  $P < 0.001$ ). In addition, rofecoxib's analgesic efficacy was similar to that of ibuprofen (TOPAR8, 18.3 vs. 17.0;  $P = 0.460$ ), but the duration was longer ( $P < 0.05$ ); with ibuprofen, the time to onset was 24 minutes, peak pain relief score was 2.9, and duration of analgesic effect was

8.9 hours. The safety profile was similar across all treatment groups. Thus rofecoxib provided analgesic efficacy superior to that of celecoxib and comparable to that of ibuprofen in the treatment of patients with acute postoperative dental pain.

Publication Types:

- Clinical Trial
- Randomized Controlled Trial

PMID: 10566562 [PubMed - indexed for MEDLINE]

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## Current concepts in the etiology and treatment of primary dysmenorrhea.

Dawood MY.

Primary dysmenorrhea may affect as many as 40 percent of all adult women, temporarily disabling one-tenth of them. The etiology of this condition may be related to excess production of prostaglandins by the endometrium following decline in progesterone levels consequent to corpus luteum regression. It is proposed that increased prostaglandin levels produce increased myometrial contractility and uterine ischemia and sensitization of pain fibers, resulting in pelvic pain. Administration of nonsteroidal anti-inflammatory agents which block the cyclooxygenase enzyme of the arachidonic acid cascade is an effective treatment for primary dysmenorrhea, as is oral contraceptive therapy. Criteria for an ideal prostaglandin synthetase inhibitor are described.

Publication Types:

- Review

PMID: 3548208 [PubMed - indexed for MEDLINE]

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## Dysmenorrhea.

**Dawood MY.**

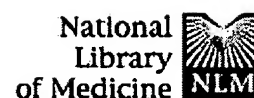
Dysmenorrhea affects over 50% of menstruating women and causes extensive personal and public health problems, a high degree of absenteeism and severe economic loss. In primary dysmenorrhea there is no macroscopically identifiable pelvic pathology, while in secondary dysmenorrhea gross pathology is present in the pelvic structures. With primary dysmenorrhea the pain is suprapubic and spasmodic, and associated symptoms may be present. Characteristically dysmenorrhea starts at or shortly after menarche. The pain lasts for 48-72 hours during the menstrual flow and is most severe during the first or second day of menstruation. It is now clear that in many women with primary dysmenorrhea the pathophysiology is due to increased and/or abnormal uterine activity because of the excessive production and release of uterine prostaglandins. Treatment with many of the prostaglandin synthetase inhibitors (nonsteroidal antiinflammatory drugs) will produce significant relief from dysmenorrhea and a concomitant decrease in menstrual fluid prostaglandins. For dysmenorrheic women who desire oral contraception, this agent will relieve the dysmenorrhea by suppressing endometrial growth, thus resulting in a decrease in the menstrual flow as well as in menstrual fluid prostaglandins. For those not requiring oral contraception the drug of choice for primary dysmenorrhea remains a prostaglandin inhibitor. Laparoscopy need be resorted to only if a pelvic abnormality is detected on examination or if treatment with prostaglandin inhibitors for up to six months is not significantly effective. In secondary dysmenorrhea, relief is obtained when the pelvic pathology--such as ovarian cysts, uterine fibroids, adhesions, cervical stenosis, congenital malformation of the uterus and endometriosis--is treated. In women using IUDs the dysmenorrhea is readily controlled with prostaglandin inhibitors since the underlying pathophysiology is excessive prostaglandin production and release.

PMID: 3158737 [PubMed - indexed for MEDLINE]

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☐ 1: Contraception 1999 Nov;60(5):269-74

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**ELSEVIER SCIENCE  
FULL-TEXT ARTICLE**

## Comparison of efficacy, cycle control, and tolerability of two low-dose oral contraceptives in a multicenter clinical study.

**Endrikat J, Dusterberg B, Ruebig A, Gerlinger C, Strowitzki T.**

Department for Gynecology and Obstetrics, University of Heidelberg, Germany. jan.endrikat@schering.de

This study compares the contraceptive reliability, cycle control, and tolerability of two oral contraceptive preparations containing 20 micrograms of ethinyl estradiol combined with either 75 micrograms of gestodene (EE/GSD) or 150 micrograms of desogestrel (EE/DSG). Women received the trial preparations daily for 21 days, followed by a 7-day pill-free interval. Contraceptive efficacy, cycle control, and tolerability were evaluated over a period of 12 cycles. Efficacy data of 14,700 treatment cycles (EE/GSD: 7299; EE/DSG: 7401) were obtained from 1476 women (EE/GSD, n = 740; EE/DSG, n = 736). Both preparations provided effective contraception and good cycle control with a similarly low incidence of both spotting and breakthrough bleeding. The spotting rates in both treatment groups decreased from 35.1% (EE/GSD) and 37.5% (EE/DSG) in the first treatment cycle to approximately 10% in the fourth treatment cycle. The spotting incidence as percent of the total number of cycles was 12.7% for EE/GSD and 14.3% for EE/DSG. The breakthrough bleeding incidence was 5.2% of all cycles for EE/GSD and 6.0% of all cycles for EE/DSG. For 84.7% of the cycles in the gestodene group and for 82.5% of the cycles in the desogestrel group, neither spotting nor breakthrough bleeding were recorded. Overall, the spotting and breakthrough bleeding incidence tended to be lower with EE/GSD than with EE/DSG. However, the difference was not statistically significant. Amenorrhea was recorded in 2.7% of the cycles with EE/GSD and in 2.9% with EE/DSG. Both preparations were well tolerated and showed a similar pattern of adverse events. More than 83% of the women in both groups either did not gain weight or lost more than 2 kg. Both preparations had a beneficial effect on dysmenorrhea. Both regimens provided reliable contraception and good cycle control. The incidence of adverse events was relatively low and both preparations were well tolerated.

Publication Types:

- Clinical Trial
- Multicenter Study
- Randomized Controlled Trial

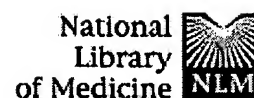
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## Primary dysmenorrhea.

**Coco AS.**

Family Practice Residency, Lancaster General Hospital, Pennsylvania 17604, USA.

Primary dysmenorrhea is defined as cramping pain in the lower abdomen occurring just before or during menstruation, in the absence of other diseases such as endometriosis. Prevalence rates are as high as 90 percent. Initial presentation of primary dysmenorrhea typically occurs in adolescence. It is a common cause of absenteeism and reduced quality of life in women. The problem is often underdiagnosed and undertreated. Women with primary dysmenorrhea have increased production of endometrial prostaglandin, resulting in increased uterine tone and stronger, more frequent uterine contractions. A diagnostic evaluation is unnecessary in patients with typical symptoms and no risk factors for secondary causes. Nonsteroidal anti-inflammatory medications are the mainstay of treatment, with the addition of oral contraceptive pills when necessary. About 10 percent of affected women do not respond to these measures. It is important to consider secondary causes of dysmenorrhea in women who do not respond to initial treatment. Many alternative treatments (ranging from acupuncture to laparoscopic surgery) have been studied, but the supporting studies are small, with limited long-term follow-up.

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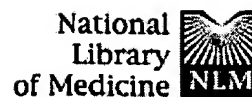
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☐ 1: Ann N Y Acad Sci 2000;900:237-44

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## Dysmenorrhea.

**Deligeoroglou E.**

2nd Department of Obstetrics and Gynecology, University of Athens  
Aretaieion Hospital, Greece. [geocre@aretaieio.uoa.gr](mailto:geocre@aretaieio.uoa.gr)

Dysmenorrhea presents as painful periods that start two to three years after menarche. The pain usually begins when the bleeding starts and lasts for 48-32 hours. The cause of menstrual cramps and associated symptoms in primary dysmenorrhea is related to prostaglandin production. In secondary dysmenorrhea, there is documented pelvic pathology that causes the painful menstrual cramps, and treatment is cause related. Available treatments for primary dysmenorrhea--NSAIDS, oral combined contraceptives, beta-blockers, psychotherapeutic methods, and cervical dilatation--are discussed.

### Publication Types:

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# Primary Dysmenorrhea

ANDREW S. COCO, M.D., Lancaster General Hospital, Lancaster, Pennsylvania

Primary dysmenorrhea is defined as cramping pain in the lower abdomen occurring just before or during menstruation, in the absence of other diseases such as endometriosis. Prevalence rates are as high as 90 percent. Initial presentation of primary dysmenorrhea typically occurs in adolescence. It is a common cause of absenteeism and reduced quality of life in women. The problem is often underdiagnosed and undertreated. Women with primary dysmenorrhea have increased production of endometrial prostaglandin, resulting in increased uterine tone and stronger, more frequent uterine contractions. A diagnostic evaluation is unnecessary in patients with typical symptoms and no risk factors for secondary causes. Nonsteroidal anti-inflammatory medications are the mainstay of treatment, with the addition of oral contraceptive pills when necessary. About 10 percent of affected women do not respond to these measures. It is important to consider secondary causes of dysmenorrhea in women who do not respond to initial treatment. Many alternative treatments (ranging from acupuncture to laparoscopic surgery) have been studied, but the supporting studies are small, with limited long-term follow-up. (Am Fam Physician 1999;60:489-96.)

Primary dysmenorrhea is a very common problem in young women. It is usually defined as cramping pain in the lower abdomen occurring at the onset of menstruation in the absence of any identifiable pelvic disease. It is distinguished from secondary dysmenorrhea, which refers to painful menses resulting from pelvic pathology such as endometriosis. A relative lack of physician awareness of the very high rates of prevalence and the substantial morbidity of dysmenorrhea often leads to inadequate treatment of this problem. With the widespread availability of over-the-counter nonsteroidal anti-inflammatory drugs (NSAIDs), it is often assumed that women are treating themselves adequately. Unfortunately, this is not always the case.

## Epidemiology

Primary dysmenorrhea is by far the most common gynecologic problem in menstruating women. It is so common that many women fail to report it in medical interviews, even when their daily activities are restricted. Reported prevalence rates are as high as 90 percent.<sup>1</sup> A recent prospective study of college students, based on diaries kept for one year,

found that 72 percent of monitored periods were painful, most commonly during the first day of menses. Sixty percent of the women studied reported at least one episode of severe pain.<sup>2</sup>

The problem of absenteeism from school or work is also underappreciated. In one study<sup>2</sup> of college women, 42 percent of the study subjects reported absenteeism or loss of activity on at least one occasion, although only a small percentage of women missed work or school for a given monthly menstrual cycle. In several longitudinal studies of young women, rates of absenteeism ranged from 34 to 50 percent.<sup>3,4</sup> In an older study,<sup>5</sup> dysmenorrhea accounted for 600 million lost work hours and \$2 billion in lost productivity annually.

The study<sup>2</sup> concluded that several risk factors were associated with more severe episodes of dysmenorrhea: earlier age at menarche, long menstrual periods, smoking, obesity and alcohol consumption. Other studies have not found an association with obesity or alcohol, and these issues remain controversial.<sup>3,4,6</sup> Another report, using a cross-sectional sample of 1,147 urban adolescents, showed that attempting to lose weight was significantly associated with increased

Primary dysmenorrhea usually presents during adolescence. Pain begins within hours of the onset of menstruation and peaks in the first day or two of the cycle.

#### Menstrual Fluid Prostaglandin Levels

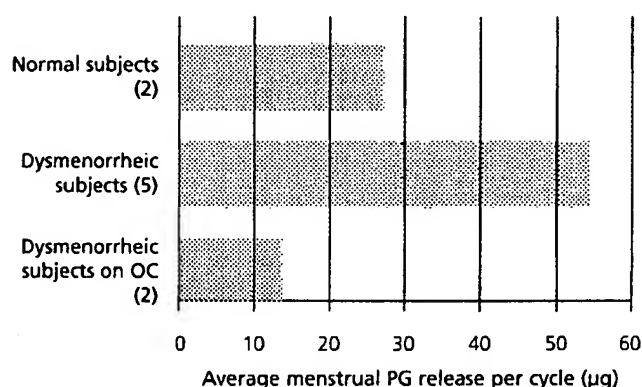


FIGURE 1. Menstrual fluid prostaglandin levels in normal and dysmenorrheic subjects with and without oral contraceptives. (OC = oral contraceptives; PG = prostaglandin)

Adapted from Chan WY, Dawood MY. Prostaglandin levels in menstrual fluid of nondysmenorrheic and of dysmenorrheic subjects with and without oral contraceptive or ibuprofen therapy. *Adv Prostaglandin Thromboxane Leukotriene Res* 1980;8:1443-7.

menstrual pain.<sup>7</sup> Physical activity was not associated with pain characteristics.

Data to substantiate the widely held view that menstrual pain diminishes after child-bearing are inconsistent. In one longitudinal study,<sup>4</sup> there was evidence of a decreased prevalence and severity of dysmenorrhea after parity, but other studies found no such effect.<sup>8,9</sup> Overall, these epidemiologic studies provide some information for patient education efforts. The potential for decreasing painful periods may provide sufficient motivation for some women to adopt healthy lifestyle changes, such as smoking cessation.

#### Etiology

The etiology of primary dysmenorrhea is not precisely understood, but most symptoms can be explained by the action of uterine prostaglandins, particularly  $\text{PGF}_{2\alpha}$ . During endometrial sloughing, the disintegrating endometrial cells release  $\text{PGF}_{2\alpha}$  as menstruation begins.  $\text{PGF}_{2\alpha}$  stimulates myometrial contractions, ischemia and sensitization of nerve endings. The clinical evidence for this theory is quite strong. Women who have more severe dysmenorrhea have higher levels of  $\text{PGF}_{2\alpha}$  in their menstrual fluid (Figure 1). These levels are highest during the first two days of menses, when symptoms peak. In

TABLE 1

#### Circumstances That May Indicate Secondary Dysmenorrhea

1. Dysmenorrhea occurring during the first one or two cycles after menarche (congenital outflow obstruction).
2. Dysmenorrhea beginning after 25 years of age.
3. Late onset of dysmenorrhea after a history without previous pain with menstruation (consider complications of pregnancy: ectopic or threatened spontaneous abortion).
4. Pelvic abnormality on physical examination; infertility (consider endometriosis, pelvic inflammatory disease or other causes of scarring); heavy menstrual flow or irregular cycles (consider adenomyosis, fibroids, polyps); dyspareunia.
5. Little or no response to therapy with nonsteroidal anti-inflammatory drugs, oral contraceptives, or both.

Information from references 9 and 11.

addition, numerous studies have documented the impressive efficacy of NSAIDs, which act through prostaglandin synthetase inhibition.<sup>10</sup> Some studies have also implicated increased levels of leukotrienes and vasopressin, but these connections are not yet well established.

### Clinical Presentation and Diagnosis

Primary dysmenorrhea usually presents during adolescence, within three years of menarche.<sup>11</sup> It is unusual for symptoms to start within the first six months after menarche. Affected women experience sharp, intermittent spasms of pain, usually centered in the suprapubic area. Pain may radiate to the back of the legs or the lower back. Systemic symptoms of nausea, vomiting, diarrhea, fatigue, fever, headache or lightheadedness are fairly common. Pain usually develops within hours of the start of menstruation and peaks as the flow becomes heaviest during the first day or two of the cycle.

A focused history and physical examination are usually sufficient to make the diagnosis of primary dysmenorrhea. The history reveals the typical cramping pain with menstruation, and the physical examination is completely normal. Secondary causes of dysmenorrhea must be excluded. *Table 1* lists some of the circumstances in which the diagnosis of secondary dysmenorrhea should be considered. *Table 2* lists selected causes of secondary dysmenorrhea.

Some secondary causes may be differentiated by inquiring about age of menarche, length of cycle, and the regularity and timing of the pain. It is usually possible to differentiate dysmenorrhea from premenstrual syndrome (PMS) based on the patient's history. The pain associated with PMS is generally related to breast tenderness and abdominal bloating, rather than a lower abdominal cramping pain. PMS symptoms begin before the menstrual cycle and resolve shortly after menstrual flow begins. Endometriosis may present as progressive dysmenorrhea but is

*If pain fails to respond to therapy with nonsteroidal anti-inflammatory drugs, the diagnosis of primary dysmenorrhea should be reconsidered.*

often accompanied by pain during intercourse and may affect fertility.

In addition to the history of the timing of pain, the patient's family history may be helpful in differentiating endometriosis from primary dysmenorrhea. Endometriosis has been found in up to 7 percent of first-degree relatives of women with confirmed endometriosis compared with an approximate overall incidence of 1 percent in the general population.<sup>12,13</sup> An early diagnosis of endometriosis during adolescence can be an important step in minimizing the long-term sequelae, including pain and infertility.

A detailed sexual history is essential to assess for the risk of pelvic inflammatory disease (PID). Women with a previous history of PID, sexually transmitted diseases, multiple sexual partners or unprotected sex are at increased risk.

The physical examination centers on the bimanual pelvic examination. Findings during the nonmenstrual phase of the cycle are typically negative. If the pain is reproducible,

**TABLE 2**  
**Possible Causes of Secondary Dysmenorrhea**

<i>Uterine causes</i>	<i>Extrauterine causes</i>
Adenomyosis	Endometriosis
Pelvic inflammatory disease	Inflammation and scarring (adhesions)
Cervical stenosis and polyps	Functional ovarian cysts
Fibroids (intracavitary or intramural)	Benign or malignant tumors of ovary, bowel or bladder, or other site
Intrauterine contraceptive devices	Inflammatory bowel disease

*Information from Smith RP. Gynecology in primary care. Baltimore: Williams & Wilkins, 1997:389-404.*

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*Oral contraceptives may be up to 90 percent effective in relieving the pain of primary dysmenorrhea.*

it should be nonspecific and limited to the midline. The primary intent of the examination is to rule out secondary causes of pain such as tumors or ovarian cysts.

With a typical history and a lack of abnormal findings on routine pelvic examination, further diagnostic evaluation is not needed. In fact, in many instances, it is preferable to confirm the diagnosis through a therapeutic trial of NSAIDs. At least partial relief of pain with NSAID therapy is so predictable in women with primary dysmenorrhea that failure to respond should raise doubts about the diagnosis.

### Treatment

Most patients with primary dysmenorrhea show subjective improvement with NSAID treatment.<sup>10</sup> Various studies report successful pain relief in 64 to 100 percent of subjects.<sup>9</sup> These familiar drugs have a record of efficacy demonstrated by numerous studies over the past 15 years. Oral contraceptives provide another effective and well-studied choice for therapy, especially in women desiring birth control. Oral contraceptives are effective in about 90 percent of patients with primary dysmenorrhea.<sup>8</sup> For the approximately 10 percent who do not respond to these options,

a host of alternatives exists, ranging from laparoscopic surgery to acupuncture, although with much less evidence to support their use. Again, lack of pain relief should increase suspicion of a secondary cause of dysmenorrhea. See Figure 2 for a suggested treatment algorithm.

### NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

The most appropriate first-line choice of therapy in most women with primary dysmenorrhea is an NSAID. These medications work through the inhibition of the production and release of prostaglandins. As mentioned previously, prostaglandins are responsible for the painful uterine contractions and associated systemic symptoms of primary dysmenorrhea, such as nausea and diarrhea. The choices of specific agents are numerous, and no particular NSAID has been reliably shown to be more effective than others for this condition. Note that aspirin is not used for the treatment of dysmenorrhea. It is not potent enough in the usual dosage. Response to NSAIDs usually occurs within 30 to 60 minutes. Since individual response may vary, it may be prudent to try a second agent of a different class if the pain is not relieved with the first agent after one or two menstrual cycles.

Although NSAIDs are highly effective and widely available without a prescription, many adolescents are not utilizing effective treatment regimens.<sup>14</sup> In one study,<sup>14</sup> 25 percent of adolescents used less than the recommended dosage of medications, and 43 percent failed to reach the maximum daily frequency. Clinicians should routinely inquire about the use of over-the-counter drugs, including specific dosing, especially with young patients.

### ORAL CONTRACEPTIVES

Oral contraceptives are the second line of therapy for most patients, unless birth control is also desired. The necessity of daily medication to prevent symptoms for one or two days a month makes them too cumber-

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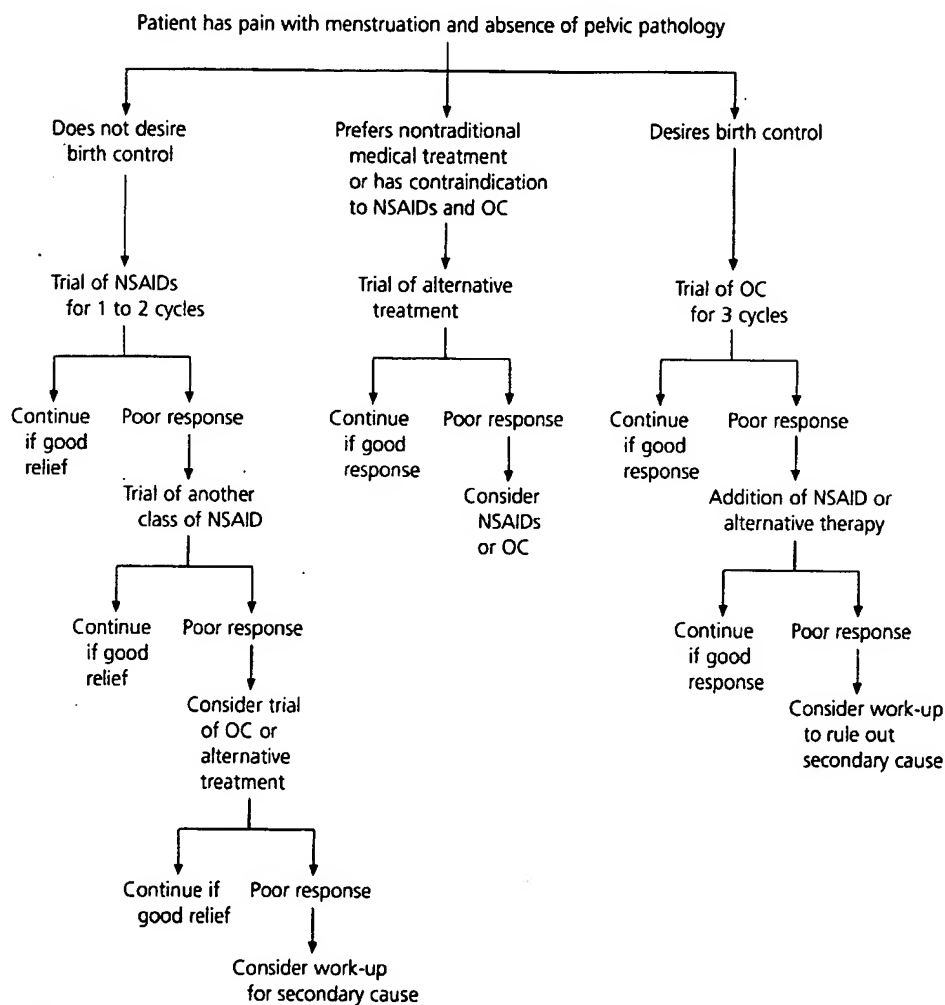
Address correspondence to Andrew S. Coco, M.D., Associate Director, Family Practice Residency, Lancaster General Hospital, 555 N. Duke St., P.O. Box 3555, Lancaster, PA 17604. Reprints are not available from the author.



some as a first-line choice compared with the highly effective NSAIDs. Oral contraceptives prevent menstrual pain through a different mechanism than NSAIDs. The action of oral

contraceptives is twofold: reduction of menstrual fluid volume and suppression of ovulation.<sup>8</sup> As mentioned previously, they are up to 90 percent effective.<sup>8</sup> Any oral contracep-

### Treatment of Primary Dysmenorrhea



**FIGURE 2.** Algorithm for treatment of patients with primary dysmenorrhea. (NSAIDs = non-steroidal anti-inflammatory drugs; OC = oral contraceptives)

Adapted from Morse A, Cullins VE. Dysmenorrhea. In: Rakel RE, ed. *Conn's Current therapy*, 1997: latest approved methods of treatment for the practicing physician. Philadelphia: Saunders, 1997:1090.

tive will work. Studies attempting to prove the superiority of triphasic preparations over monophasic medications or of one type of progesterone component over another have been largely inconclusive.<sup>15</sup> All oral contraceptives are very effective compared with placebo.

In general, it may take up to three cycles for menstrual pain to noticeably diminish, so it is important to stress this point to patients at the time of the initial prescription and consider adding an NSAID for breakthrough pain during the interim.

Many adolescents are not aware that oral contraceptives reduce menstrual pain.<sup>16</sup> In a prospective study of 308 adolescent females, those with severe symptoms of dysmenorrhea

that responded to oral contraceptives were eight times as likely to be consistent users of oral contraceptives.<sup>16</sup> Counseling patients about this added benefit might improve their motivation to comply with a daily medication. Norplant and DepoProvera are also effective in relieving dysmenorrhea. As with NSAIDs, it is important to inquire about contraindications: cardiovascular disease, cerebrovascular disease, hepatic disease, history of venous thrombosis or current pregnancy.

Because NSAIDs and oral contraceptives are so effective and work through different mechanisms, a combination of the two is a very attractive option in refractory cases. No consistent data demonstrate effectiveness rates for this combination, but it is probably

**TABLE 3**  
**Therapies For Refractory Dysmenorrhea**

Therapy	Description	Comments
TENS unit	Four small studies (126 patients total) <sup>17-20</sup>	42 to 60% of patients had at least moderate relief; less NSAID was needed in one study; TENS worked faster than naproxen in one study.
Laparoscopic presacral neurectomy	Two small studies, one using laser (88 patients total) <sup>21,22</sup>	33 to 88% effective up to 12 months after treatment.
Acupuncture	One study (43 patients followed for one year) <sup>23</sup>	Up to 91% improvement in symptoms and 41% decrease in analgesic use.
Omega-3 fatty acids	Two studies: one (181 patients) epidemiologic survey of dietary intake <sup>24</sup> and one (42 patients) using supplements as therapy <sup>25</sup>	Low dietary intake correlated with menstrual pain; treatment group had significantly lower scores on pain scale.
Transdermal nitroglycerin	One study (65 patients), 0.1 to 0.2 mg of nitroglycerin given per hour during first few days of menstrual cycle <sup>26</sup>	90% effective; 20% of patients reported headache.
Thiamine (vitamin B <sub>1</sub> )	Randomized double-blind trial (556 patients), 100 mg taken orally each day for 90 days <sup>27</sup>	87% of patients cured up to two months after treatment. Study was performed in India, so the study population might have had preexisting nutritional deficiency.
Magnesium supplements	One study (30 patients) of magnesium pidolate <sup>28</sup>	Up to 84% decrease in symptoms, especially on days 2 and 3 of menstrual cycle.

(TENS = transcutaneous electric nerve stimulation; NSAID = nonsteroidal anti-inflammatory drug)

Information from references 17 through 28.

at least 90 percent, given the previously stated rates of effectiveness for the individual treatments. Consequently, about 10 percent of patients may remain nonresponders to combination treatment.

#### ALTERNATIVE THERAPIES

For reasons that are not clear, about 10 percent of women with primary dysmenorrhea do not respond to treatment with NSAIDs or oral contraceptives. In addition, some women have contraindications to these medications. Consequently, researchers have investigated numerous alternative treatments. Table 3<sup>17-28</sup> lists therapies for nonresponders and indicates some of the studies that support their use.

Trials of transcutaneous electrical nerve stimulation (TENS) units, laparoscopic presacral neurectomy, acupuncture, omega-3 fatty acids, transdermal nitroglycerin, thiamine and magnesium all demonstrated some relief of dysmenorrhea symptoms, although the numbers in the studies were small and only short-term follow-up was noted. Women should be encouraged to try any safe option and to feel comfortable discussing these options with their physician. A survey<sup>29</sup> showed that Americans are increasingly using alternative therapies and not discussing these therapies with their physicians.

#### PATIENTS WHO DO NOT RESPOND

Women who do not respond to therapy with NSAIDs and/or oral contraceptives present a dilemma. Nonresponse is also an indication to consider some secondary cause of dysmenorrhea, such as endometriosis. One study<sup>30</sup> indicated that most women with endometriosis endure pain for many years before the condition is detected—the mean delay in diagnosis after onset of pain symptoms was almost 12 years in American women. The diagnostic dilemma arises at least partly from the need for invasive testing, i.e., laparoscopy, before the diagnosis can be reliably made.

#### Final Comment

Primary dysmenorrhea is a very common and underappreciated problem. Family physicians may need to specifically inquire about menstrual pain to identify patients who are not achieving effective treatment with the use of over-the-counter medications or alternative therapies.

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AU Djuric, Stevan W.; Huang, Fu-Chih; Regan, John R.  
CS Dept Inflammation and Bone Metabolism Chemistry, Rhone-Poulenc Rorer  
Central Research, Collegeville, PA, 19426, USA  
SO Expert Opinion on Therapeutic Patents (1994), 4(7), 785-802  
CODEN: EOTPEG; ISSN: 0962-2594  
DT Journal; General Review  
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Thank you,  
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# Patent Update

## Pulmonary-Allergy, Dermatological, Gastrointestinal & Arthritis

### Anti-inflammatory patent highlights part 2: patents from the second half of 1993

Stevan W Djuric, Fu-Chih Huang and John R Regan

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*Exp. Opin. Ther. Patents* (1994) 4(7):785-802

#### Introduction

This update provides a synoptic overview of the most significant anti-inflammatory agents to be disclosed in the patent literature during the second half of 1993. The division of subject matter for the review follows that of the earlier update for the anti-inflammatory patent literature for the first half of 1993 with the exception that a section on inhibitors of protein kinase C has been added. The sections are:

- leukotriene biosynthesis inhibitors and antagonists
- cyclooxygenase and dual 5-LO/CO inhibitors
- platelet activating factor antagonists
- inhibitors of phospholipase A<sub>2</sub>
- tachykinin antagonists
- inhibitors of tissue destructive proteinases
- cytokine synthesis inhibitors/antagonists
- phosphodiesterase inhibitors
- immunosuppressive agents
- Inhibitors of cell-cell adhesion

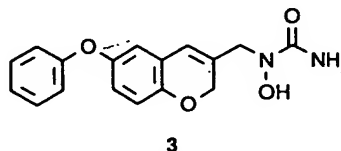
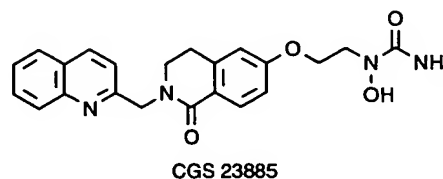
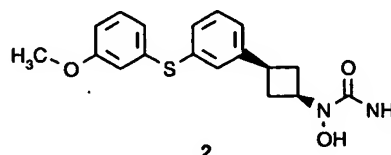
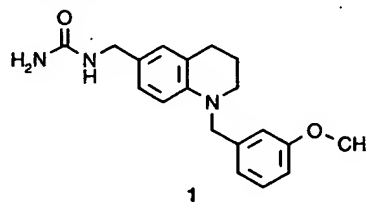
#### Leukotriene Biosynthesis Inhibitors

The search for leukotriene biosynthesis inhibitors is still one of the most active areas in the modulation of the 5-lipoxygenase (5-LO) pathway of arachidonic metabolism. Amongst different classes of compounds, hydroxamic acid and N-hydroxy urea derivatives remain popular.

Pfizer has disclosed three series of hydroxamic acids or N-hydroxyureas as 5-lipoxygenase inhibitors. Com-

pound **1** is one of the examples claimed in a US patent [101]. In a rat peritoneal resident cell assay the IC<sub>50</sub> values ranged from 0.1 to 30  $\mu$ M, for this series of compounds. Two distinct cycloalkyl hydroxyurea series have been disclosed as 5-lipoxygenase inhibitors [102, 103]. The IC<sub>50</sub> values for *in vitro* lipoxygenase inhibitory activity ranged from 0.01 to 30  $\mu$ M. Compound **2** was a typical example.

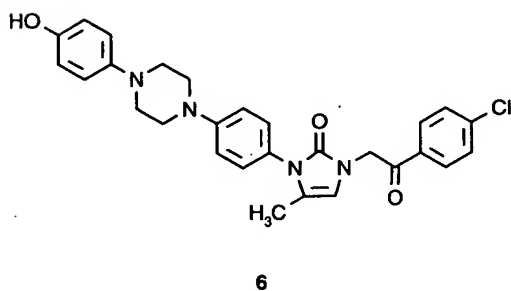
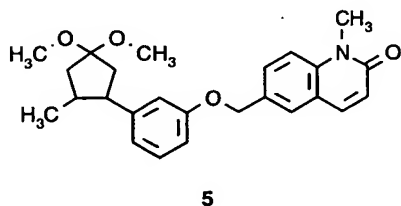
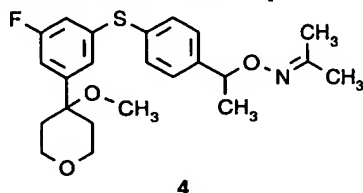
Eight isoquinolinyl substituted hydroxylamine derivatives, represented by **3**, were specifically claimed by Ciba-Geigy as 5-LO inhibitors in a US patent [104], but no biological data were presented. In a related work, a series of chromene derivatives was evaluated as 5-LO



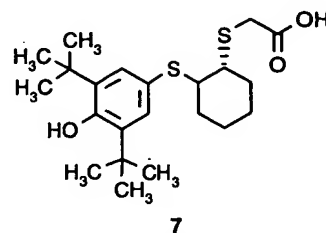
inhibitors. CGS 23885 displayed an  $IC_{50}$  of 48 nM in an A23187-stimulated 5-HETE formation assay in guinea-pig PMNs. The compound also has exhibited an  $ED_{50}$  of 0.23  $\mu\text{g/kg}$  (*po*) in an *ex vivo* dog model [1].

Zeneca has disclosed two series of 4-aryl-4-methoxytetrahydropyrans as 5-LO inhibitors [105, 106]. The inhibitory activity of these compounds was assessed using a human whole blood assay. Compound 4 had an  $IC_{50}$  of 90 nM in this paradigm; 4 had an  $ED_{50}$  of 1  $\mu\text{g/kg}$  for inhibition of  $LTB_4$  synthesis in the zymosan induced peritonitis model in the rat. A series of 1,1-dimethoxycycloalkane derivatives were also claimed as 5-LO inhibitors [107]. Two compounds were specifically claimed and the most potent compound, 5, had an  $IC_{50}$  of 40 nM.

Janssen has revealed a series of triazolone derivatives that have selective 5-LO inhibitory properties and inhibit A23187-induced  $LTB_4$  synthesis in beagles by about 80% at 1.25  $\mu\text{g/kg}$  (*po*) for up to 4 hours [108]. Compound 6 was the only specifically claimed compound and no *in vitro* data were provided.

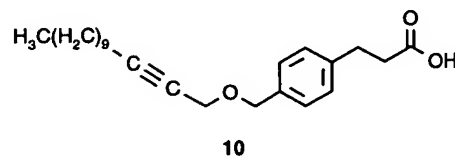
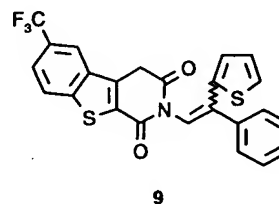
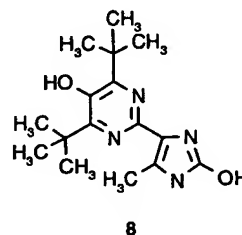


Phenolic ethers are commonly claimed as 5-LO inhibitors. In this context, Searle has disclosed a series of phenolic thioethers [109]. Three compounds were specifically claimed; 7 inhibited 5-LO and superoxide generation with  $IC_{50}$  values of 1.1  $\mu\text{M}$  and 3  $\mu\text{M}$ , respectively.



## Dual 5-LO/Cyclooxygenase Inhibitors

The search for dual 5-LO and cyclooxygenase (CO) inhibitors continued to be an interesting approach towards the identification of novel anti-inflammatory agents. Two Warner-Lambert patents related to pyrimidines disclosed dual 5-LO and CO inhibitors [110, 111]. In a whole cell assay, 8 inhibited 5-LO and CO with  $IC_{50}$  values of 1.8 and 1.35  $\mu\text{M}$ , respectively. A series of N-alkenylbenzo(3,2-*b*)oxazin-2,4-diones from Pfizer represent structurally different dual 5-LO and CO inhibitors [112]. These compounds, including 9, were stated to be active in *in vivo* inflammatory models but no data were provided, however.

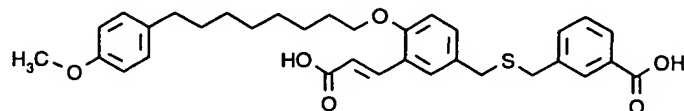


## 12-Lipoxygenase Inhibitors

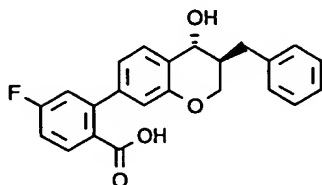
A series of aryl aliphatic acids have been disclosed by Wayne State University as 12-lipoxygenase inhibitors [113]; however, the specificity was not reported. Nine compounds with  $IC_{50}$  values ranging from 0.035  $\mu\text{M}$  to 5  $\mu\text{M}$  were listed. Compound 10 was one of the examples claimed.

## Leukotriene Receptor Antagonists

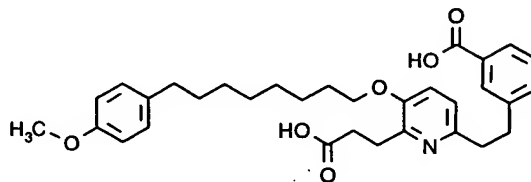
Although there is increasing evidence to suggest that, the pro-inflammatory lipoxygenase derived metabolite,  $LTB_4$  may play an important role in many inflammatory disease states, its specific role in human



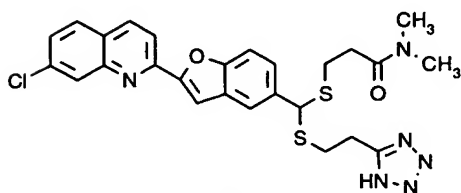
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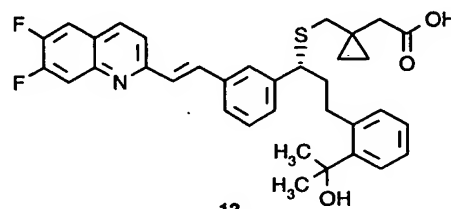
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SB 201044



12



13

inflammatory disease remains elusive. Thus, efforts have continued in the search for potent antagonists. SmithKline Beecham has published the biological profile of SB 201993 [2]. The compound was a competitive LTB<sub>4</sub> receptor antagonist with a K<sub>i</sub> of 7.1 nM on human PMNs. The SAR of a series of trisubstituted pyridines has also been communicated by the same group [3]. Lilly continued to remain active in this area and has further extended their earlier work by combining structural features from different series to give new antagonists [4,5-6,7]. The best compounds in each series had IC<sub>50</sub> values less than 10 nM.

In the patent field, Pfizer has disclosed 3-substituted chromanols as LTB<sub>4</sub> receptor antagonists [114]. Compound **11** was one of two, with (3*R*,4*S*) stereochemistry as shown, claimed in this series, but no biological data were provided.

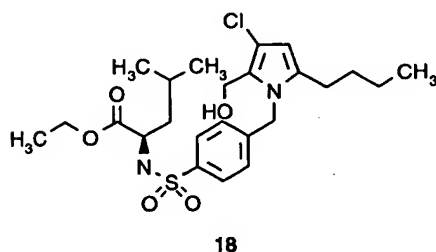
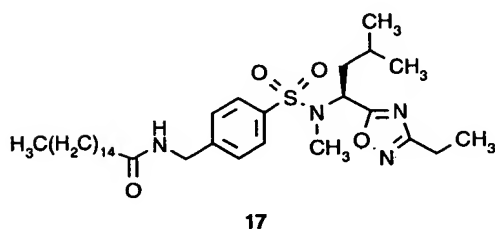
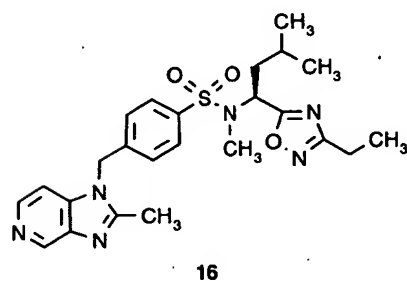
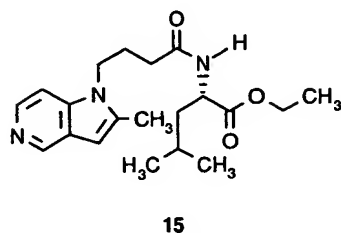
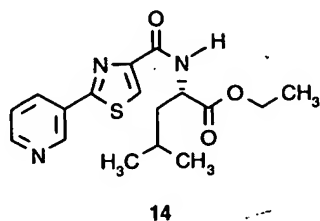
SB 201044 is one of five pyridylalkanoic acids claimed as LTB<sub>4</sub> antagonists by SmithKline Beecham [115]. The diacid functional groups apparently are needed for high binding affinity. This series of compounds, therefore, appears to generically resemble LTB<sub>4</sub> antagonists previously reported by Lilly. Efforts in the LTD<sub>4</sub> antagonists domain appear to have declined as judged by patent application activities. There were only two patent disclosures during this period. These patents seem to reflect on the fine tuning of existing arts. Fujisawa has disclosed a series of quinolinylbenzofurans, represented by **12**, as LTD<sub>4</sub> antagonists [116]. In addition, Merck Frosst has continued their efforts in the realm of quinoline-based antagonists. Compound

**13** was one of two compounds specifically claimed [117].

### Platelet Activating Factor Antagonists

Platelet activating factor (PAF) is postulated to be associated with many pathophysiological processes such as allergic asthma, systemic anaphylaxis, thrombosis, etc. While many selective PAF antagonists have been available for several years, the role of PAF in disease states and the therapeutic benefits of antagonists remain to be demonstrated. That there is still significant interest in finding new PAF antagonists, however, is evidenced by patent activities. British Bio-Tech has disclosed a series of five related patents. In the first, which highlighted an N-acyl leucine series [118], four compounds were specifically claimed. These compounds inhibited <sup>3</sup>H-PAF binding to human platelet plasma membranes with IC<sub>50</sub>s of 20-30 nM; **14** was one of the examples. Modification of the N-acyl group, while maintaining the leucine backbone, appeared to improve potency [119]. For example, **15** exhibited an IC<sub>50</sub> of 3 nM in the binding assay. This compound also inhibited PAF-induced hypotension in anaesthetised rats with an ED<sub>50</sub> of 30 µg/kg (*iv*). Further modifications of the leucine derivatives, by replacement of the N-acyl linkage and the carboxylate function with an N-sulphonyl function and oxadiazole respectively, lead to a new chemical series along with improved potency. For example, the sulphonamide derivative **16** had an IC<sub>50</sub> of 0.5 nM in the PAF binding assay [120]. Further fine tuning of the lipophilic region

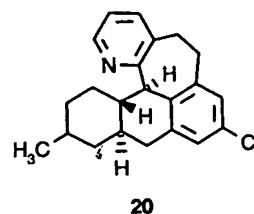
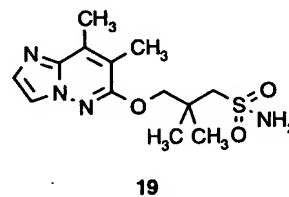




lead to another patent [121]. Compound 17, with an  $IC_{50}$  of 1 nM, was one of 57 compounds specifically claimed. Interestingly, some of the arylsulphonamide derivative of leucine were found to be both PAF and angiotensin II antagonists [122]. Specifically, 18 had an  $IC_{50}$  of 5 nM in the PAF binding assay and its N-methyl free acid has a  $pK_b$  of approximately 6.0 against angiotensin II.

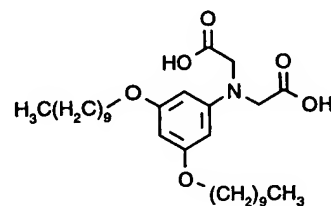
Takeda, one of the earliest players in this area, has claimed a series of imidazopyridazines as PAF antagonists [123, 124]. Some of these compounds, including 19, inhibited PAF-induced bronchoconstriction in the

canine in the range of 70-90% at a dose of 10  $\mu$ g/kg. No *in vitro* binding data were provided; however, Schering shows continued interest in compounds with dual activity as  $H_1$  and PAF antagonists [125]. Five specific pentacyclic pyridine derivatives, represented by 20, are claimed. Example 20 had an  $IC_{50}$  of 38  $\mu$ M and a  $pA_2$  value of 8.85 in the antihistamine assay.

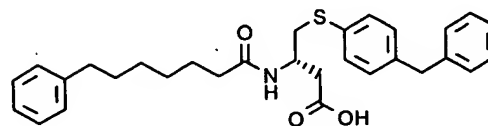


### Phospholipase A<sub>2</sub> Inhibitors

Phospholipase A<sub>2</sub> (PLA<sub>2</sub>) catalyses the hydrolysis of fatty acids from the sn-2 position of membrane phospholipids to yield fatty acids and lysophospholipids. These products may themselves function as second messengers or can be metabolised to form a variety of pro-inflammatory lipid mediators. Because of this pivotal role, there has been considerable efforts expended in the search for potent PLA<sub>2</sub> inhibitors as novel anti-inflammatory agents. Recently, significant progress has been made in this regard, particularly for type II PLA<sub>2</sub> (14kD) inhibitors. For example, Ro 23-9358 inhibits crude human synovial fluid PLA<sub>2</sub> and purified recombinant human placental PLA<sub>2</sub> with  $IC_{50}$ s of 0.23  $\mu$ M and 0.0087  $\mu$ M, respectively. In contrast, the compound is a weak inhibitor of cPLA<sub>2</sub> (85kD) from the human monocyte tumour cell line U937 ( $IC_{50}$  = 48  $\mu$ M).



Ro 23-9358

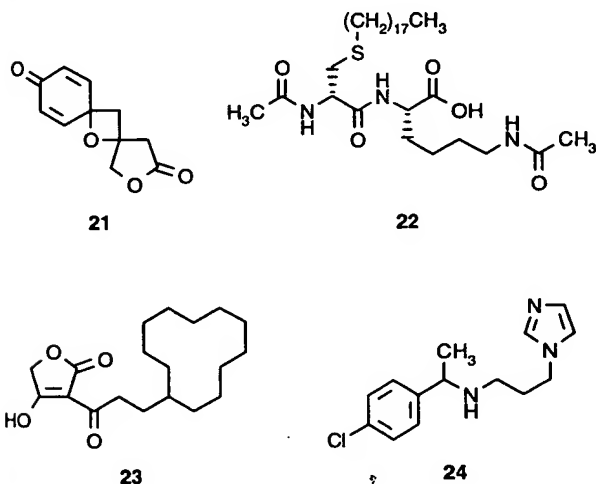


FPL 67047XX

Interestingly, Ro 23-9358 is also active in *in vivo* inflammation models [8]. FPL 67047XX is another new potent human cPLA<sub>2</sub> inhibitor with an IC<sub>50</sub> of 21  $\mu$ M. The design of the inhibitor was based on the hypothesis that the carboxylic acid group may function as a bioisosteric replacement for a phosphodiester group, and thereby provide interactions with the active site of the enzyme [9].

In the patent field, Boehringer Mannheim has claimed spiro-oxetanes as type II PLA<sub>2</sub> inhibitors. Specifically, **21** inhibited human recombinant type II PLA<sub>2</sub> by 93 % at a concentration of 100  $\mu$ g/ml [126]. A series of cysteinyl-lysine derivatives have been claimed as PLA<sub>2</sub> inhibitors. Twenty compounds related to **22** were claimed; **22** inhibited PLA<sub>2</sub> with an IC<sub>50</sub> of 15  $\mu$ M [127].

American Home Products has disclosed a series of cyclic  $\beta$ -diketones as PLA<sub>2</sub> inhibitors. The best compound inhibited PLA<sub>2</sub> by 79% at a concentration of 10  $\mu$ M. Fourteen compounds, including **23**, were specifically claimed [128]. Boots has claimed a series of imidazole derivatives as PLA<sub>2</sub> inhibitors. Compound **24**, one of 15 specifically claimed compounds, exhibited an IC<sub>50</sub> of 55  $\mu$ M [129].



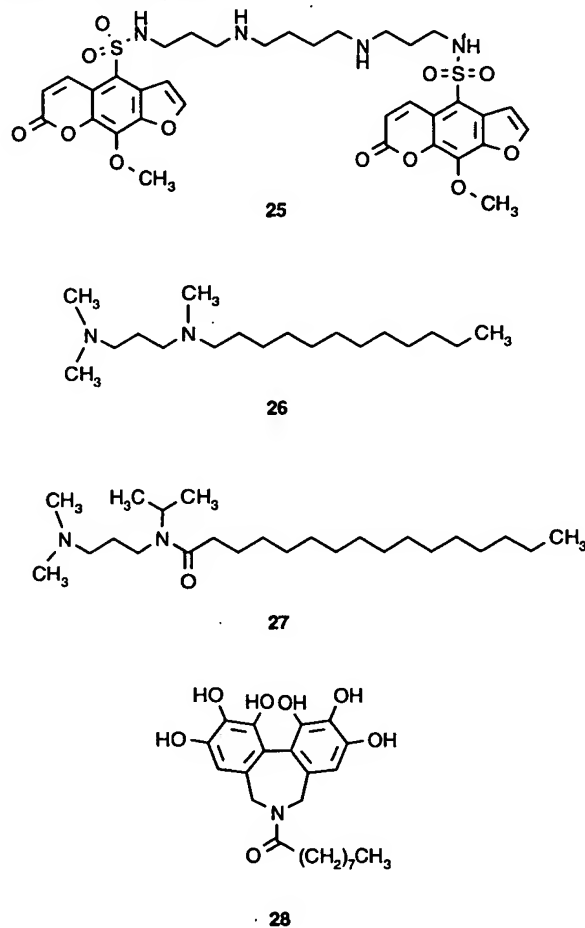
### Inhibitors of Protein Kinase C

Protein kinase C (PKC) consists of a family of isoenzymes that differ in structure, cofactor requirement and substrate specificity. To date, ten different PKC isoenzymes have been identified in different species, tissues and cell lines [10]. Potent and selective inhibitors of PKC have been shown to block activation of human T cells *in vitro* and *in vivo*. It is in this context, therefore, that it has been suggested that inhibition of PKC might represent a rational target mechanism for a new class of therapeutic agents targeted at T-cell driven chronic inflammatory or auto-immune diseases [11].

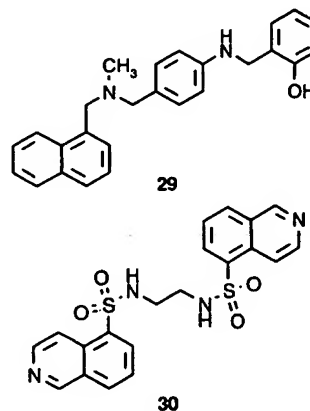
Sphinx Pharmaceuticals has lead the field in terms of number of patent applications in the PKC inhibition

area during 1993. During the second half of 1993 four applications arose from the Sphinx stable [130,131-132,133]. Compounds **25** through **28** are illustrative examples from the individual patents.

The compounds of the inventions were evaluated *in vitro* for inhibition of PKC (IC<sub>50</sub>s = 10-100  $\mu$ M), and for human tumour cell growth inhibition (MCF-7 cell line, IC<sub>50</sub>s = 0.6-6.0  $\mu$ M).



One patent from Sandoz [134] describing a series of substituted benzylamine derivatives as inhibitors of protein kinase C is of interest. Compounds of the invention are exemplified by **29**.

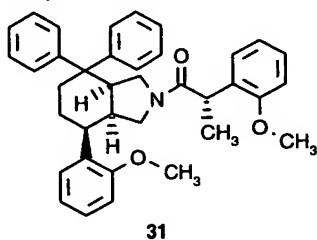


Disappointingly, no biological data were given although a description of the PKC assay was!

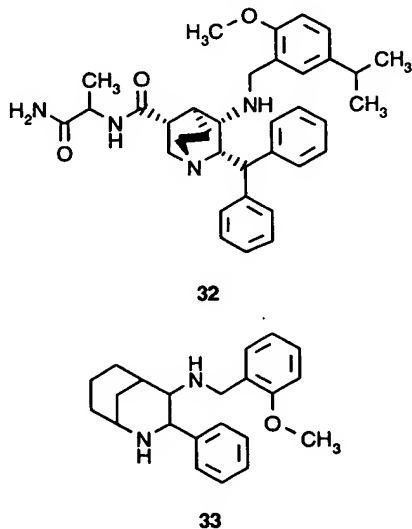
Italfarmaco has disclosed a series of isoquinoline sulphonamide derivatives as PKC inhibitors [135]. IC<sub>50</sub>s for compounds of the invention, including **30**, were in the  $\mu$ M range.

### Tachykinin Antagonists

Substance P antagonists are currently under investigation as anti-asthmatic agents and for the treatment of inflammatory pain. Merck, Pfizer and Rhône-Poulenc Rorer continue to dominate the patent literature in this speciality area. Rhône-Poulenc Rorer has disclosed a series of substance P antagonists, such as **31**, characterised by a perhydroisoindole nucleus [136].



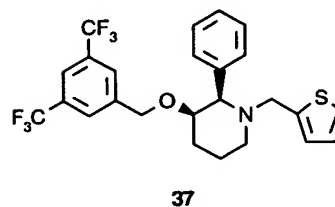
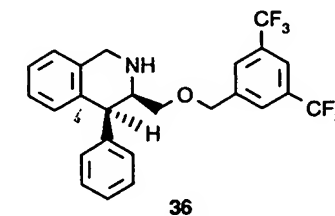
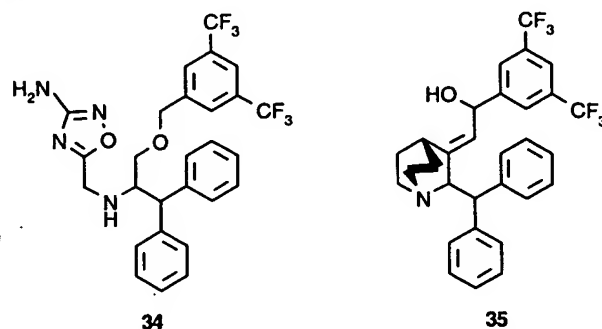
The *in vivo* activity of the specified compound was determined in guinea-pigs. Anti-inflammatory activity in substance P induced hypotensive animals was in the range of 0.04 to 3.5  $\mu$ g/kg and the ED<sub>50</sub> for formaldehyde induced pain was 11  $\mu$ g/kg.



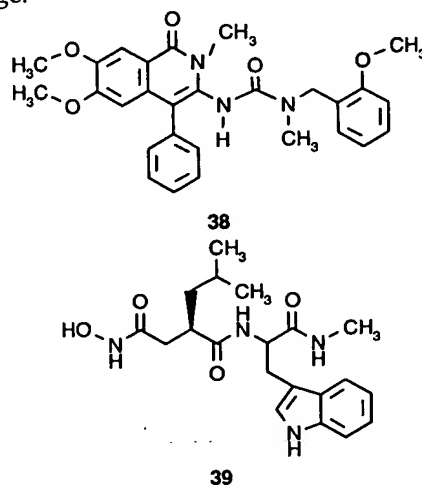
Pfizer has continued to elaborate upon its flagship antagonist CP 96,345 in two separate applications [137,138]. Compounds **32** and **33** are illustrative.

Merck has four separate patent disclosures for the second half of 1993 [139-142]. They continue to build on their already sizeable substance P empire with several novel classes of antagonists. Compounds **34** through **37** are representative examples from the four

applications. The compounds were screened for their ability to displace labelled substance P from human NK<sub>1</sub> receptors *in vitro* [143]. Typical IC<sub>50</sub> values were below 100 nM, although little data were presented.



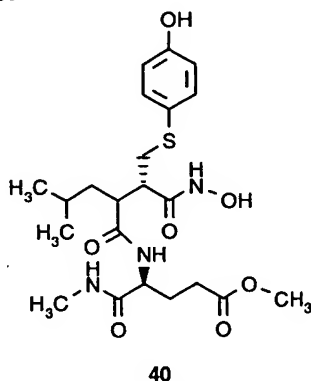
Takeda has disclosed a novel series of isoquinolones as tachykinin antagonists [144]. These compounds, exemplified by **38**, were assayed for antagonism of substance P binding to a rat forebrain homogenate (Neuropeptides (1984) 4: 325). IC<sub>50</sub>s were in the 100 nM range.



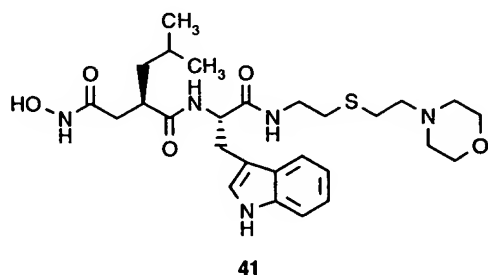
## Inhibitors of Tissue Destructive Proteinases

### MMP inhibitors

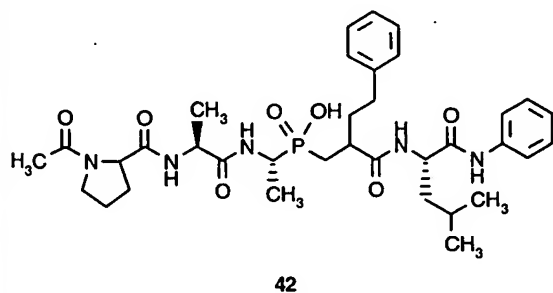
Inhibition of the matrix metalloproteinase (MMP) family of enzymes continues to be a major thrust of research within the pharmaceutical industry, as evidenced by the high volume of patent literature appearing on this topic. Reviews of this area of research have continued to appear [12a,12b] and a recent article has overviewed the development of Galardin **39**, a MMP inhibitor currently in Phase II clinical trials for corneal ulceration [13].



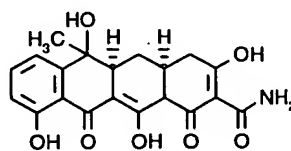
Impetus to the discovery efforts to provide rationally designed inhibitors of MMPs such as collagenase and stromelysin has recently been provided by a 2.4 Å resolution structure of the catalytic domain of fibroblast collagenase complexed with an inhibitor [14].



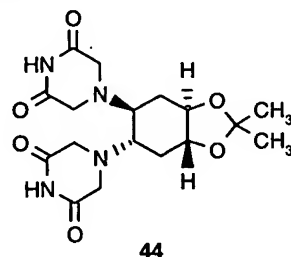
The majority of patent applications are still characterised by hydroxamic acid based inhibitors. British Bio-Tech has disclosed compounds, exemplified by **40**, as inhibitors of collagenase with IC<sub>50</sub>s in the low nM range [145].



Sterling Winthrop has elaborated on a series of succinamide derived matrix metalloproteinase inhibitors, wherein **41** is a representative example from their case [146].

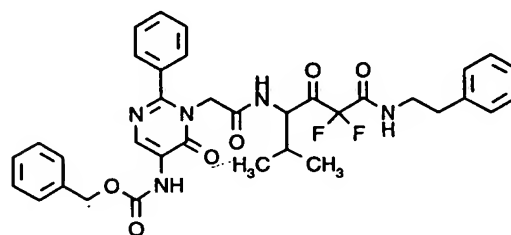
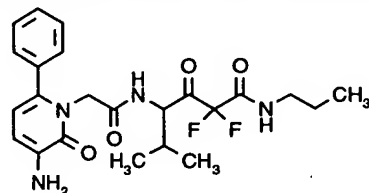


The compound exhibited an IC<sub>50</sub> of 15 nM against human fibroblast collagenase. Merck has disclosed a series of phosphonic acid derivatives as inhibitors of stromelysin and gelatinase [147]. Compound **42** had K<sub>i</sub> values of 6.1 and 2.6 nM, respectively, against the above enzymes.



The scientific literature is replete with examples of tetracyclines acting as direct inhibitors of collagenases [15] and the Kurarau Company has disclosed novel treatment methods for rheumatoid and osteoarthritis employing tetracycline derivatives such as **43** [148].

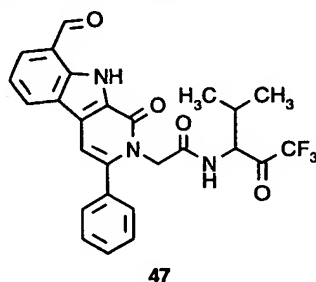
Compound **43** displayed an IC<sub>50</sub> of 2-5 μM for inhibition of collagenase and was shown to be effective in suppressing cartilage destruction in a rabbit osteoarthritis model (*Arthritis Rheum* (1983) **26**:875). Oncologix Inc has claimed a utility for MMP inhibitors in the treatment of vascular leakage syndrome [149]. Compounds of the invention are characterised by lack



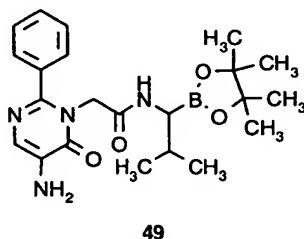
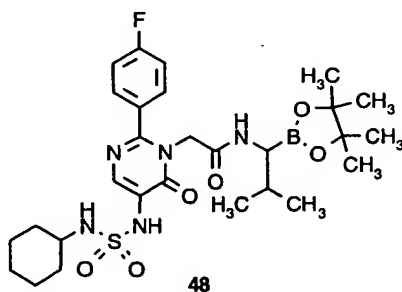
of a hydroxamic acid or phosphonic acid group. Example 44 had an  $IC_{50}$  of 8  $\mu M$  against collagenase.

### Elastase inhibitors

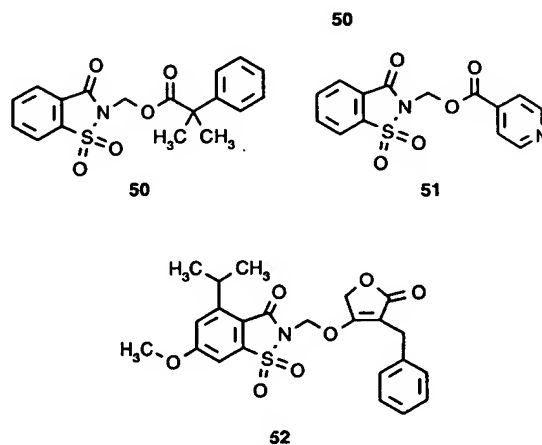
A comprehensive review of the recent developments in the area of synthetic inhibitors of human leukocyte elastase (HLE) has recently been published [16]. Zeneca, Merck and Sterling Winthrop continue to monopolise the patent domain in this highly contested area of research. Zeneca have disclosed three distinct series of fluoroacetamide containing inhibitors of HLE. [149-151] Compounds of all three inventions exemplified by **45**, **46** and **47** exhibited  $K_i$  values of less than 100 nM and were effective *in vivo* inhibitors of elastase induced lung injury in hamsters.



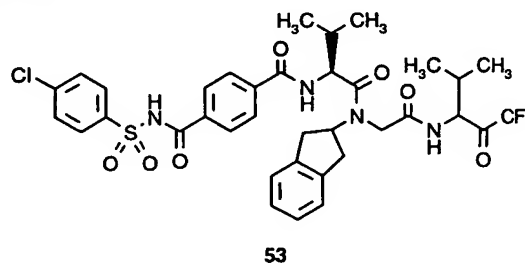
The Zeneca group has also disclosed the use of  $\alpha$ -aminoboronic acid containing peptides as elastase inhibitors in two separate applications [153-154]. Compounds of the inventions exhibited  $K_i$ s < 100 nM; **48** and **49** are representative examples.



Sterling has continued to expand its stronghold in the area of "Lazarus" type inhibitors of HLE and have disclosed a further three series of saccharin derived compounds [155-157]. Compounds such as **50**, **51** and **52** were active as inhibitors of HLE with  $K_i$  values ranging from 0.013 to 0.92  $\mu M$  (for an assay description, see *Biochem. Pharmacol* (1975) **24**:2177).

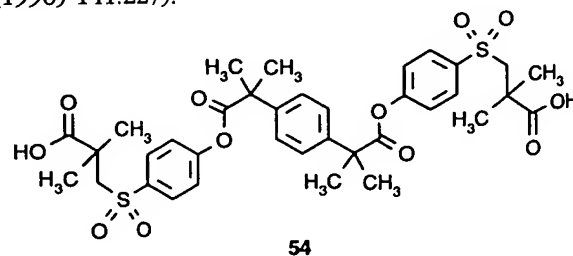


Two other patent applications are worthy of note. First, an application from the Boehringer Ingelheim group has disclosed a novel series of amide derivatives as inhibitors of HLE [158]. The compounds of the invention, such as **53**, retain the characteristic trifluoromethyl ketone functionality of this class of inhibitor.



Compounds of the invention were found to be effective in the elastase induced lung haemorrhage paradigm in hamsters when administered intratracheally at doses of 20  $\mu g/ml$  3 to 5 minutes prior to elastase administration. Secondly, Cortech has disclosed a novel series of phenylene dialkanoate ester inhibitors of human neutrophil elastase (HNE) [159].

Compounds of the invention, e.g. **54** have  $IC_{50}$  values for HNE of 0.018 to 8.44  $\mu M$ . They were evaluated in an acute respiratory distress syndrome model (AARD (1990) 141:227).



### Cytokine synthesis inhibitors/antagonists

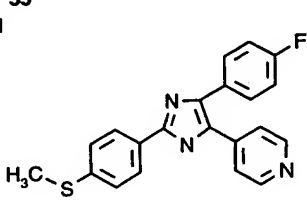
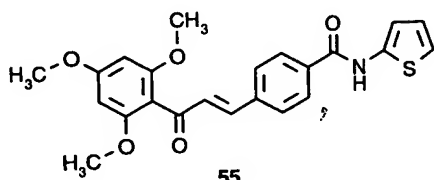
The goal of developing agents which are potent and specific inhibitors of the production of pro-inflammatory cytokines remains an attractive therapeutic target. In addition to the chronic inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease

and psoriasis, septic shock has been identified as a cytokine-mediated pathophysiological disturbance. The success of treating animal models of human inflammatory diseases with soluble cytokine receptor antagonists points to the feasibility of achieving similar results with low molecular weight cytokine biosynthesis inhibitors.

The role of cytokines in an ovalbumin-sensitised guinea-pig model of allergic lung disease was examined by the use of a recombinant interleukin-1 receptor antagonist (IL-1ra) [17]. Aerosol treatment of animals with IL-1ra immediately before antigen challenge provided significant protection against bronchial hyperactivity and pulmonary eosinophil accumulation. This study supported the concept that IL-1 plays a key role in pulmonary changes observed during allergic airway diseases.

The endogenous nucleoside 5'-deoxy-5'-methylthioadenosine (MTA) was shown to inhibit the production of TNF, but not IL-1, in LPS-activated macrophages [18]. In addition, MTA selectively inhibited the expression of ICAM-1 in endothelial cells stimulated with IL-1. It was suggested that MTA might have anti-inflammatory properties.

A group of 2'-substituted chalcone derivatives were described by Batt *et al.* that potently inhibited the release of IL-1 from human peripheral monocytes stimulated with LPS [19]. The enone group was mandatory for biological activity, implying these compounds act as alkylating agents. Compound 55 inhibited IL-1 biosynthesis *in vitro* with an IC<sub>50</sub> value of 0.3  $\mu$ M. In a model of septic shock 55, at a dose of 600  $\mu$ g/kg, po, provided a 50% survival of mice injected with LPS.



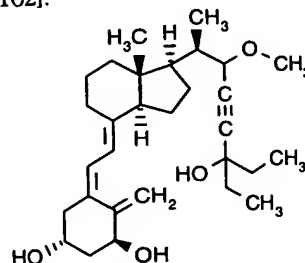
56

57

A series of aryl substituted imidazoles 56 and 57 have been claimed by SmithKline Beecham as inhibitors of cytokine production [160,161]. In human monocytes challenged with LPS compounds of type 56 furnished

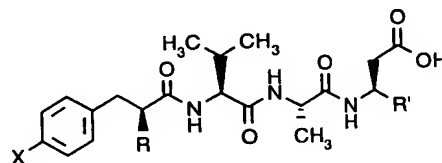
IC<sub>50</sub> values of 0.1-5 and 0.5-3.5  $\mu$ M for the prevention of IL-1 and TNF production, respectively.

Leo Pharmaceutical Products has disclosed that vitamin D<sub>3</sub> analogues with inversion of the natural configuration at C-20, e.g. 58, are selectively more potent towards the inhibition of interleukins than calcium metabolism [162].



58

Three patent applications by Merck & Co have described peptidyl inhibitors of interleukin-1 $\beta$  converting enzyme (ICE) [20]. Compounds 59 [163], 60 [164], and 61 [165] are mechanism based inhibitors containing active site-reactive functionality. They are reported to have K<sub>i</sub> values below 1  $\mu$ M. In addition, Mijalli *et al.* reported that tetrapeptide ketone 62 was a potent inhibitor of ICE with a K<sub>i</sub> = 18 nM [21].



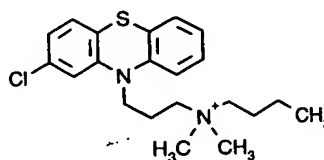
59 R = H; R' = COCH<sub>2</sub>OCOC<sub>6</sub>H<sub>4</sub>-2,6-(CF<sub>3</sub>)<sub>2</sub>

60 R = AcNH; R' = COCHN<sub>2</sub>

61 R = AcNH; R' = CN

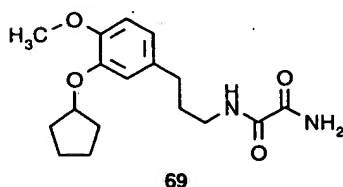
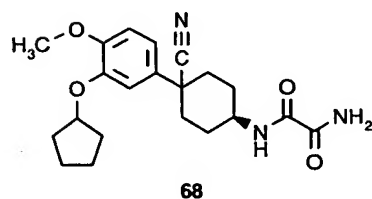
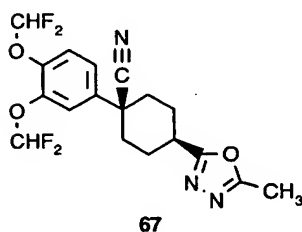
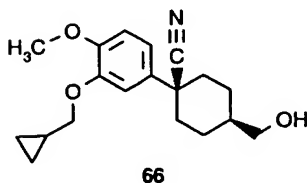
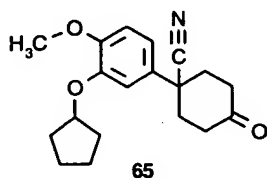
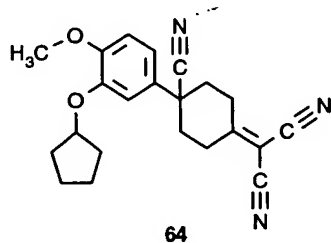
62 R = AcNH; R' = CO(CH<sub>2</sub>)<sub>5</sub>Ph; X = OH

A report by Ulich *et al.* [22] demonstrated that when the recombinant human TNF soluble receptor type I (sTNFrI) was co-injected with LPS intratracheally to rats the number of neutrophils in bronchoalveolar lavage specimens was significantly reduced compared with the injection of LPS alone. The sTNFrI was as equipotent to IL-1ra as an inhibitor of acute inflammation. No synergistic effects of a combination of IL-1ra and sTNFrI were observed in these experiments. Adir *et al.* reported that tetra-alkyl amino phenothiazine derivatives, e.g. 63, inhibited the formation of TNF by 95%



63

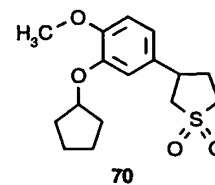
when administered to a LPS-treated mouse at a dose of 5  $\mu\text{g/kg}$  (*ip*). Survival rates were improved. The compounds are observed only in the periphery and therefore not sedating. The compounds also inhibited PLA<sub>2</sub> [166].



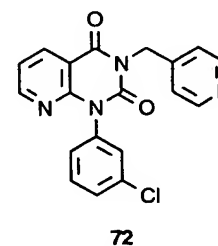
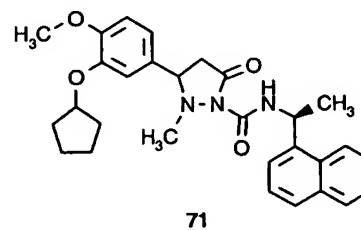
A series of aryl and nitrile substituted cyclohexanes were disclosed by SmithKline Beecham as inhibitors of phosphodiesterase IV (PDE IV) and consequently the release of TNF. Compound 64, a cyclohexyl malononitrile derivative, demonstrated a positive *in*

*in vivo* response in reducing endotoxin-induced increases in serum levels of TNF [167]. Compounds of type 65 inhibited PDE IV with IC<sub>50</sub> values in the  $\mu\text{M}$  range [168]. Phenylcyclohexylmethyl alcohol, e.g. 66, displayed IC<sub>50</sub> values in the  $\mu\text{M}$  range in an assay that measured increases in cAMP accumulation in intact tissue [169]. Phenylcyclohexyl-oxadiazole derivatives, exemplified by 67, were described as inhibitors of TNF production *in vitro* using human monocytes and *in vivo* in two endotoxin induced shock models [170]. Phenylcyclohexyloxamido derivatives, e.g. 68, were shown to inhibit TNF production *in vitro* using human monocytes. PDE IV activity was demonstrated in U937 cells in the presence of PGE<sub>2</sub> [171]. Opened ring variants of oxamide 68, e.g. 69, were disclosed to inhibit PDE IV and activate cAMP levels in U937 cells with EC<sub>50</sub> values of 0.3-10  $\mu\text{M}$  [172].

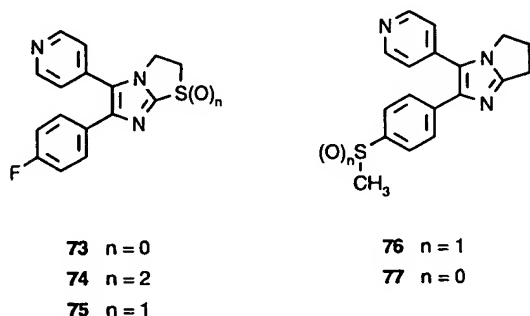
A series of phenylthiopyran derivatives, e.g. 70, were reported by Rhône-Poulenc Rorer to be selective PDE IV and TNF inhibitors [173]. IC<sub>50</sub> values against PDE IV, superoxide production in guinea-pig eosinophils and TNF production in human monocytes are between 1-10,000 nM.



American Home Products has described a series of pyrazolidinones as bronchodilators and anti-inflammatory agents by virtue of their ability to selectively inhibit PDE IV. Compound 71 is claimed to have an IC<sub>50</sub> = 12 nM against canine tracheal muscle [174]. Syntex reported that a group of pyridopyrimidinediones, e.g. 72, were inhibitors of PDEIV [175].



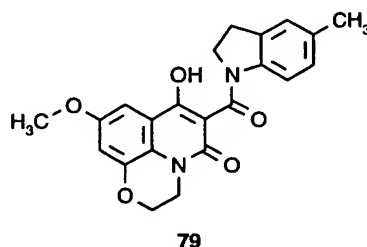
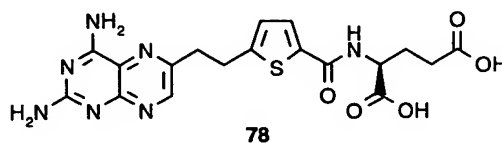
An updated review on a series of bicyclo imidazoles as cytokine biosynthesis inhibitors, as well as their modulation of arachidonic acid metabolism, has been published [23]. The *in vitro* and *in vivo* effects of SK&F 86002, **73**, its metabolite SK&F 104343, **74**, SK&F 86096, **75**, SK&F 105809, **76**, and SK&F 105561, **77**, were described. Compound **73** inhibited the *in vitro* production of IL-1 and TNF in LPS stimulated monocytes with  $IC_{50} = 0.5 \mu M$ . Sulphoxide **75** and sulphide **77** were nearly 5-10 fold less effective than **73** in this assay; sulphoxide **76** was inactive. The *in vivo* efficacy of these compounds against TNF production was assessed in LPS stimulated (*ip* injection) Balb/c mice. The doses required to inhibit 50% of TNF release was 32, 17 and 48  $\mu g/kg$ , *po*, for compounds **73**, **74** and **76**, respectively. Mechanistically, it was determined that **73** selectively inhibits a step close to the time of onset of TNF mRNA translation.



Synergen has claimed that for the prevention and treatment of IL-1 and TNF-mediated diseases a combination of recombinant IL-1 and TNF inhibitors provides beneficial synergistic effects [176]. Lewis rats injected with SCW and LPS were treated singularly with IL-1 or TNF inhibitors and a 16% decrease in swelling was observed. A combination of inhibitors provided a 41% reduction in swelling. Synergistic effects were demonstrated in models of ARDS, endotoxaemia and sepsis.

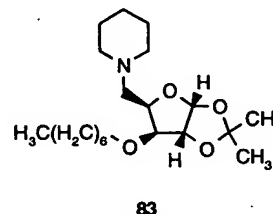
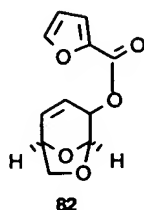
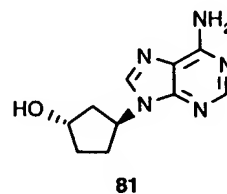
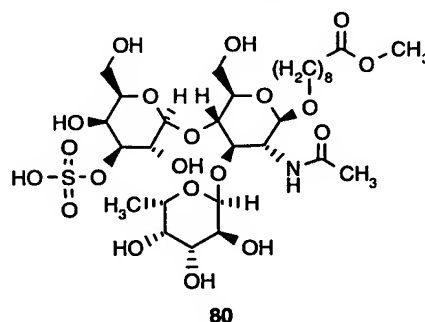
A study on the ability of antioxidants to inhibit IL-1, TNF and IL-6 production in human mononuclear cells was reported [24]. It had been observed that antioxidants inhibit the activation of two of the transcription factors (NF- $\kappa B$  and AP-1) required for inducing expression of cytokine genes. The antioxidants butylated hydroxyanisole, tetrahydropapaveroline (THP), apomorphine, norapomorphine, nordihydroguaiaretic acid and mepacrine inhibited the *in vitro* production of cytokines in LPS stimulated human peripheral blood mononuclear cell cultures with  $IC_{50}$  values ranging from 1.0–3.0  $\mu M$ . In contrast, ascorbic acid, tocopherol, mannitol, trolox, butylated hydroxytoluene, and quercetin were ineffective in this assay at doses of 50–200  $\mu M$ . *In vivo* results of some of the antioxidants ability to inhibit cytokine production was described. Mice were treated with either THP or apomorphine before

and after LPS challenge. Both compounds were effective inhibitors of IL-1 production at doses of 50  $\mu g/kg$ , *sc*.



### Immunosuppressive Agents

Several compounds with different molecular structures have been claimed to be immunosuppressant agents with unknown mechanisms of action. Seven are described here. SRI International has claimed compound **78** as possessing anti-inflammatory activity against mice challenged with type-II collagen [176]. Tricyclic heterocycles, e.g. **79**, were described by Fujisawa Pharmaceutical Co to inhibit graft-host disease and lung tumours at a dose of 32  $\mu g/kg$  [177].

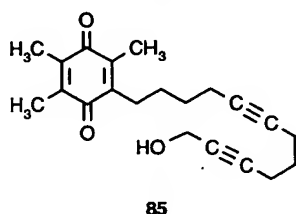
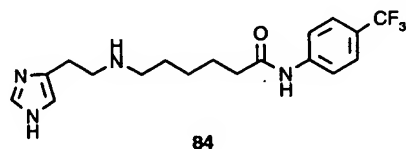


Alberta Research Council have described oligosaccharides, e.g. **80**, related to blood group determinants, as

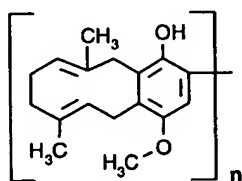


possessing the ability to suppress the secondary immune response in inflammatory action [178]. The compounds were assessed in several delayed-type hypersensitivity reaction models in rats and mice. Marion Merrell Dow claimed *trans*-cyclopentanyl analogues as immunosuppressants. Compounds of type **81** reduced the expression of MHC II antigen on rat macrophages *in vitro* [179].

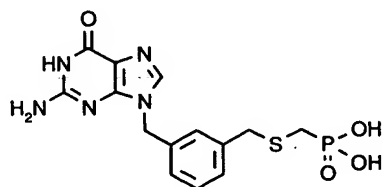
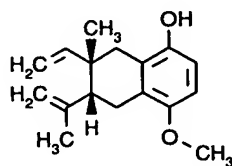
Immunosuppressive activity was claimed by Ishira Sangyo Kaisha Ltd for a series of enopyranose derivatives, e.g. **82**. At doses of 50 µg/kg enopyranose compounds provided significant reduction of arthritis in a collagen-induced mouse model of inflammation [180]. Greenwich Pharmaceutical has described 5- or 6-deoxyhexose monosaccharide having a saturated nitrogen containing heterocycle; **83** is exemplified. IC<sub>50</sub> values 30 µM were obtained in models of inflammation [181]. Histamine derivatives, e.g. **84**, were claimed to have immunomodulating activity by Immunologic Pharmaceutical Corp [182].



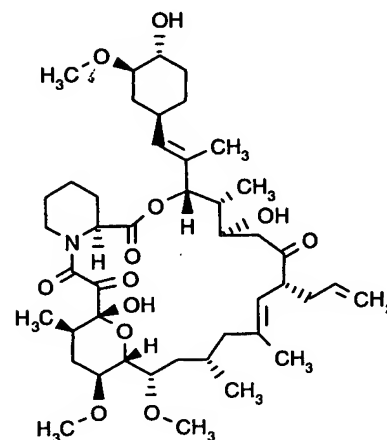
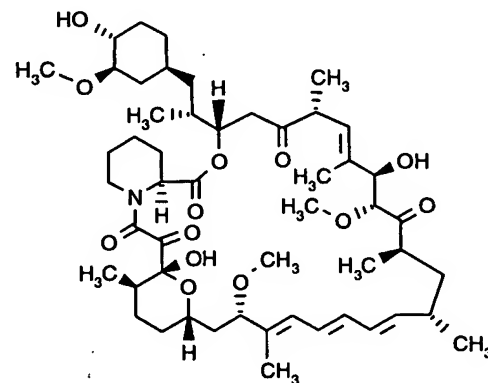
The 5-lipoxygenase inhibitor, AA-861, **85**, was shown to be an effective agent for reducing liver allograft rejection in rats. At a dose of 20 µg/kg/day, *sc*, **85** suppressed the elevation of 5-LO products and in-



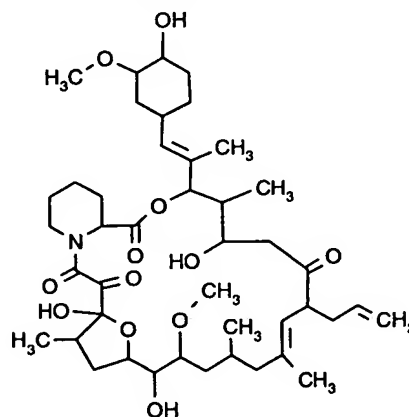
**87** n = 2



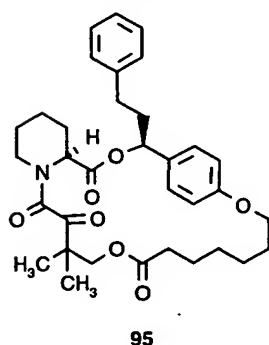
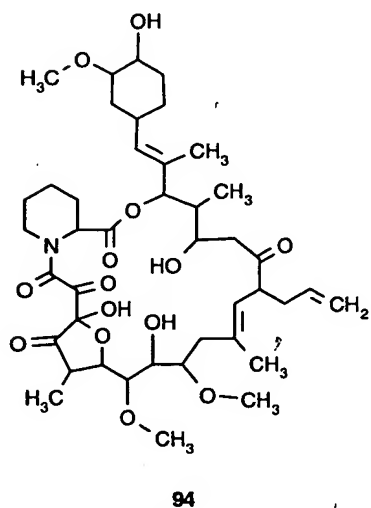
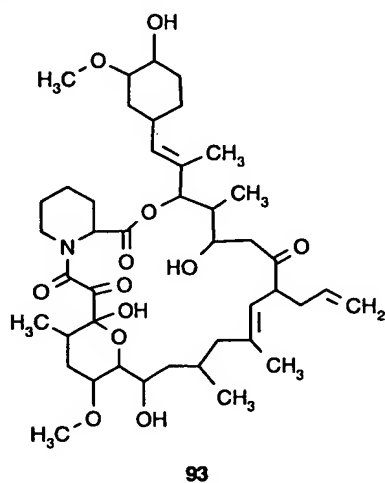
creased PGE<sub>2</sub> production in the early stage of rejection [25].



The benzothiazepine calcium antagonists diltiazem and clentiazem were shown to improve survival in heterotopic rat heart transplants. When administered with cyclosporin these two calcium channel blockers acted synergistically to increase survival time [26]. Three components of a mushroom, *lactarius flavidulus*, have shown a suppressive effect on the prolifera-



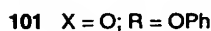
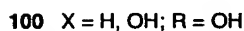
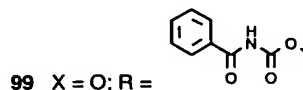
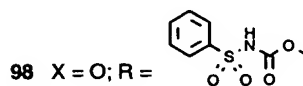
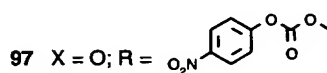
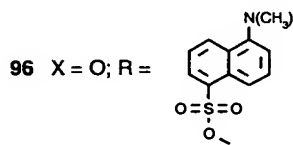
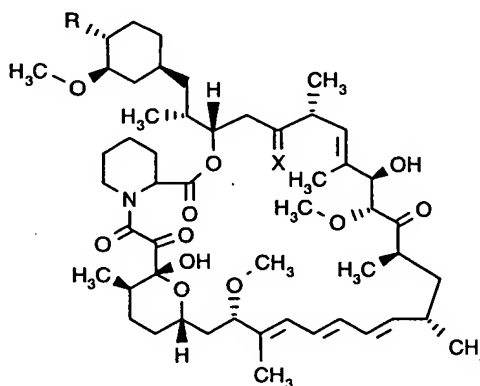
tion of mouse lymphocytes stimulated with Con A and LPS. The  $IC_{50}$  values for the three geranylphenols **86-88** against LPS stimulated lymphocytes were 7, 28 and 4  $\mu\text{g/ml}$ , respectively [27]. A series of cyclic peptides from mushrooms were described as possessing immunosuppressive activity [28]. In PFC and DTH assays cycloamanide A [c-(Phe-Phe-Ala-Gly-Pro-Val-)] and its D-Phe containing analogue were the most potent. A collection of 9-(phosphoalkylbenzyl) guanines were reported to be potent inhibitors of human erythrocyte purine nucleoside phosphorylase (PNPase) [29]. Compound **89** inhibited PNPase with a  $K_i = 1 \text{ nM}$ .



Two total syntheses of rapamycin **90** have recently been reported [30,31]. The biological basis for the actions of **90** [32], FK-506 **91** and cyclosporin [33] have been reviewed.

Oxidative metabolites of **90** from *in vitro* incubation with rat hepatic microsomes have been identified [34]. Compound **92** was observed as the major metabolite and **93** and **94** as minor ones.

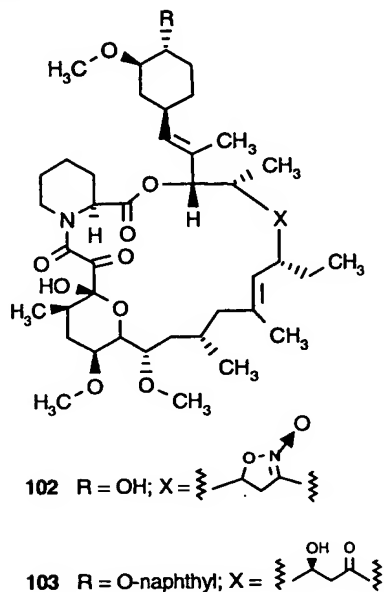
Simplified analogues of **91** were described as high affinity FKBP ligands [35]. Macrocyclic **95**, in which the pyranose and oxidised cyclohexylethyl domains were replaced with unfunctionalised aliphatic and phenethyl groups respectively, produced a  $K_i, \text{app} = 1 \text{ nM}$ . The binding mode of the macrocycle with FKBP12 was determined by X-ray crystallography to be identical with that observed for the crystalline state of both **90** and **91**.



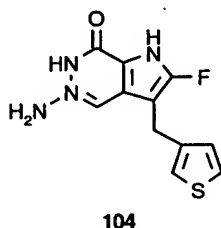
American Home Products has disclosed several C-42 rapamycin derivatives. The compounds claimed include naphthalene sulphonate ester **96** [183], phenylcarbonate **97** [184], phenylsulphonylcarbamic esters **98** [185] and benzoylcarbamic esters **99** [186]. In addition

C-27 deoxy and C-27 hydroxyrapamycin derivatives, e.g. **100**, were described [187]. In an *in vitro* lymphocyte proliferation test, the IC<sub>50</sub> values for these compounds were equivalent to rapamycin. Merck has disclosed C-42 O-alkyl, O-aryl, O-alkenyl and O-alkynylrapamycin derivatives, e.g. **101**, as agents for the treatment of autoimmune diseases [188]. In a T-cell proliferation assay compound **101** was active at 1 µg/ml.

Two disclosures for derivatives of macrolides have appeared. Fisons reported that **102** could be used as an immunosuppressant [189]. Merck reported O-aryl, O-alkyl, O-alkenyl and O-alkynyl-macrolides, as exemplified by **103** [190], as possessing immunosuppressive activity.



A series of 8-hydroxy-9-deazaguanine derivatives were described to inhibit PNPase. IC<sub>50</sub> values of compounds of type **104** were between 0.9-3.2 µM for the conversion of <sup>14</sup>C-inosine to <sup>14</sup>C-hypoxanthine in human erythrocytes [191].



### Inhibitors of Cell-Cell Adhesion

The cellular adhesion of Chinese hamster ovary cells, transfected with human CD4, and human B lymphoblastoid cell lines expressing class II molecules has been described [36]. It was reported that this adhesion process is a late event and energy dependent. ATP is required for the establishment and maintenance of

stable CD4/class II-mediated cell conjugates. The role of CD4 in T-cell adhesion regulation was discussed.

A new receptor-like protein tyrosine phosphatase (receptor-PTP) was recently cloned. This transmembrane protein is believed to contribute to signalling in cell-cell recognition [37]. The ectodomain includes an Ig-like and four fibronectin type-III-like domains. The cloned protein dramatically promoted cell-to-cell adhesion in a haemophilic calcium-independent manner.

Platelet/endothelial cell adhesion molecule-1 (PECAM-1) is an integral membrane glycoprotein expressed on endothelial cells, platelets and leukocytes. The ligand interactions of PECAM-1 involved in aggregation process were described [38]. PECAM-1 contains a glycosaminoglycan consensus binding sequence in the second Ig-like domain. PECAM-1 mediated cellular aggregation was inhibited by heparin, suggesting that this interaction involved glycosaminoglycans on adjacent cells. No aggregation occurred in those cells expressing mutant PECAM-1 protein missing the important second domain. Aggregation could also be inhibited in the presence of synthetic peptides mimicking the consensus glycosaminoglycan binding sequence.

Eli Lilly has disclosed novel oligosaccharides that bind to cell adhesion receptors. Recombinant human protein C was treated with glycanase and the enzymatically released oligosaccharides were purified chromatographically. These oligosaccharides inhibited cell adhesion *in vitro* at concentrations below 50 µM [192]. Nippon Shinyahu described Sle-type oligosaccharides containing noramoline [193]. Fred Hutchinson Cancer Research Center reported that certain tripeptides inhibit the adhesion of lymphocytes to the endothelium. The tripeptide Leu-Asp-Val was disclosed as a minimal peptide capable of supporting lymphocyte adhesion via the α-4-β-1 extracellular matrix receptor [194].

Biogen has described treatments for inflammatory bowel disease [195] and asthma [196] with the use of a monoclonal antibody that recognises integrin VLA-4 (very late antigen-4). Most types of white blood cells and tissues in the gut express VLA-4. In an animal model of IBD an effective dose of the anti-VLA-4 antibody significantly reduced acute inflammation as observed in biopsied intestinal tissue.

The Research and Development Institute described antibodies specific for multiple adhesion molecules [197]. The raised antibody, EL-246, was shown to block the function of human E-selectin and L-selectin. In an animal model of ischaemia EL-246 provided a 100% survival rate. A monoclonal antibody, IG9, was raised against the monocyte adhesion protein [198]. IG9 was

specific for the monocyte adhesion molecule and did not correspond to VCAM or ELAM. IG9 was injected intraperitoneally into a rabbit along with IL-1. The levels of inflammation and monocyte adhesion protein in the cells was reduced compared to controls administered with IL-1 alone.

Cytel Corp have claimed a specific antibody to P-selectin [199] which binds to functional epitopes on P-selectin and blocks adhesion of leukocytes. The antibody, PBL-3, was raised in a RBF/DnJ male mouse. When tested *in vivo* on rats with acute lung injury PBL3 reduced haemorrhage by 37%.

Centocor reported that peptides having as their core portions the 58-61 amino acid sequence of P-selectin were inhibitors of selectin binding [200]. Peptides contained the general formula R-X-Pro-Gln-Ser-Thr-Y-R. The peptides inhibited the adhesion of human neutrophils to purified P-selectin at concentrations between 50-1500  $\mu$ M. Oligonucleotides, between 15-20 mers, were claimed as antisense oligonucleotides capable of inhibiting the expression of Cathepsin G by Centocor [201]. The inhibition of this protease would aid in the suppression of cytotoxic lymphocyte action in graft versus host disease after transplantation.

## Conclusions

The search for new anti-inflammatory and/or immunomodulatory agents continues with much alacrity within the pharmaceutical industry as judged by the vast patent literature that continues to appear month after month. At present, of the newer classes of therapeutic agent, 5-LO inhibitors, LTD<sub>4</sub> and LTB<sub>4</sub> antagonists are undergoing clinical trials. Definitive results with these agents are eagerly awaited. For the future, if we look into the crystal ball, we may anticipate the development of agents that inhibit the inducible isoforms of nitric oxide synthase or cyclooxygenase. We might see agents that interfere, specifically, with one of the numerous cell adhesion molecules that have been identified over the last few years and even agents that directly antagonise the complex sequence of events involved in antigen recognition. It seems clear that agents of this nature will become increasingly evident in the patent art over the next few years.

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